

TESTICULAR STEROID SECRETION IN RESPONSE TO GnRH-MEDIATED LH AND FSH RELEASE IN BULLS¹

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SUMMARY

Assay of frequently collected blood samples in four mature Hereford bulls indicated the existence of a tonic mechanism for the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), i.e., their episodic release was not observed. Increased plasma concentrations of LH and FSH, however, were obtained in response to an intravenous injection of 500 µg of gonadotropin releasing hormone (GnRH). These gonadotropins showed similar secretory profiles after GnRH, but the relative magnitude of the LH response (30-fold) was considerably greater than that of the FSH response (sevenfold).

Concentrations of testosterone in jugular and spermatic vein blood were increased sevenfold after the administration of GnRH; whereas, concentrations of estrogen increased only twofold. Both steroids reached maximum concentrations 3 hr after GnRH (approximately 1 hr after the LH and FSH peak) then returned to preinjection levels. Estrogen profiles showed considerable between animal variation as compared to the testosterone secretory profiles.

Concentrations of testosterone and estrogen in control animals were markedly higher in plasma from the spermatic vein (40 ng/ml and 14.4 pg/ml) than in plasma from the systemic circulation (1 ng/ml and 4.8 pg/ml).

It is concluded from these data that the bovine testis secretes both testosterone and estrogen and that their secretion is regulated to a certain extent by hypothalamic and hypophyseal hormones. The possibility that these steroids are secreted by separate compartments within the testes is discussed.

(Key Words: GnRH, LH, FSH, Testosterone, Estrogen, Bulls.)

INTRODUCTION

Normal reproductive function requires rather precise coordination of several related events, i.e., hypothalamic neurohormone release, hypophyseal gonadotropin secretion, gonadal steroidogenesis and target organ responses. Assay of frequently collected serial blood samples from bulls (Katongole *et al.*, 1971; Smith *et al.*, 1973; Haynes *et al.*, 1976) indicates that an important relationship exists between serum concentrations of luteinizing hormone (LH) and the secretion of testosterone. Secretion of other physiologically important steroids in response to LH stimulation has received little attention.

Circadian rhythms for the secretion of LH apparently do not exist in the bull; however, episodic release of this gonadotropin has been reported (Katongole *et al.*, 1971; Smith *et al.*, 1973; Haynes *et al.*, 1976; Thibier, 1975). Whether increased concentrations of LH occur naturally by episodic release or result from the administration of gonadotropin releasing hormone (Golter *et al.*, 1972; Zolman *et al.*, 1973; Mongkonpunya *et al.*, 1974; Mori *et al.*, 1974; Thibier, 1976), temporal increases in the concentration of blood testosterone are observed. The testis is assumed to be the major source of testosterone, but direct evidence to

¹The authors are indebted to Dr. K. Folkers, University of Texas at Austin, for synthetic gonadotropin releasing hormone; Dr. L. E. Reichert, Emory University, Atlanta, GA, for purified LH (LER-1065-C2); Dr. D. J. Bolt, USDA-ARS, Beltsville, MD, for LH antisera (DJB 3-12/11); Dr. K. W. Cheng, University of Manitoba, Winnipeg, for purified FSH and FSH antisera; and the Endocrine Study Section, NIH, Bethesda, MD, for bovine LH (NIH-LH-B8) and bovine FSH (NIH-FSH-B1) used in this study. The technical assistance of Ms. Marti Brown, Donna Taubenheim and Marilyn Bierman and cooperation of the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, is acknowledged.

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support such a conclusion is limited. Secretion of estradiol in response to gonadotropic stimulation has been shown in the rat (deJong *et al.*, 1973) and man (Mahoudeau *et al.*, 1975; Kley *et al.*, 1976) and concentrations of estrogen have been shown to increase in serum at the time of semen collection in bulls (Weathersbee and Lodge, 1976). After consideration of the gonadotropin-inhibiting qualities of estradiol in male sheep (Schanbacher and Ford, 1977) and the possibility that a functional role exists for the high concentrations of estrogen in semen (Ganjam and Amann, 1974; Eiler and Graves, 1977), the need for more information on estrogen secretion in the ruminant male becomes increasingly apparent. The present study was undertaken in an effort to determine 1) the occurrence of episodic release of LH and follicle stimulating hormone (FSH) in mature Hereford bulls, 2) the response of both LH and FSH to intravenous gonadotropin releasing hormone (GnRH) administration, and 3) the subsequent secretory profiles of testosterone and estrogen in both systemic and spermatic venous blood.

MATERIALS AND METHODS

Four mature Hereford bulls (~24 months of age) were used to investigate the temporal relationship between LH and FSH release and testicular steroid secretion. During the month of May, these animals were taken off feed and placed into individual holding pens. The following morning (day 1), each bull was given an intramuscular injection (30 mg/100 kg body weight) of Rompun (Haver-Lockart Laboratories) and situated on a tilt surgical table for subsequent cannulation of a jugular and testicular spermatic vein. After local anesthesia was induced with cyclaine, a high lateral incision was made through the scrotum and the pampiniform plexus was exposed. Vinyl tubing (1.12 mm × 1.65 mm × 2 m; Becton-Dickson) which had been previously sterilized and heparinized was inserted into the spermatic vein approximately 20 to 30 cm towards the posterior vena cava. The cannula was then sutured to the external cremaster and surrounding connective tissue and exteriorized through the incision site. A stopcock was placed on the exposed end of the cannula and secured to the tail-head region of the animal for ease of blood collection. Indwelling jugular cannulae were also inserted for subsequent collection of

systemic blood samples.

After a 24-hr recovery period, the four bulls appeared normal and no edema or inflammation was detected around the scrotal incision. These animals were subsequently assigned to and used in the following split-plot design experiment. Two animals were injected with saline (control injection) on day 2 of the experiment and with 500 µg GnRH on day 3, while another two animals received saline and GnRH injections on reciprocal days. Saline and GnRH injections were administered intravenously via the jugular cannulae at approximately 0900 hour. Three blood samples were drawn at 20-min intervals before saline or GnRH injection so that baseline concentrations of LH and testosterone could be determined. Sampling at 20-min intervals continued 4 hr post-injection and then at hourly intervals for an additional 4 hours. Cannulae remained patent throughout the experiment by periodically flushing with heparinized saline (500 IU/ml). Blood samples were kept on ice until plasma could be separated by centrifugation; all samples were stored at -20 C until hormone levels were determined by radioimmunoassay.

Plasma concentrations of LH were determined by a modification of the double antibody radioimmunoassay for bovine LH described by Niswender *et al.* (1969) and reported for ovine LH by Echterkamp *et al.* (1976). The antiserum to ovine LH, at a working dilution of 1:40,000, bound 45 to 50% of the ¹²⁵I-LH and produced parallel inhibition curves with NIH-LH-B8 reference standards (.2 to 35 ng) and steer plasma. Cross-reactivity with NIH bovine preparations of prolactin, FSH, thyroid stimulating hormone and growth hormone resulted in parallel inhibition curves equal to the LH contamination of these hormone preparations. All plasma samples were assayed in duplicate within a single assay and the coefficient of variation for all duplicates was less than 10%.

A double antibody radioimmunoassay described by Cheng (1978) was used with slight modification to quantify plasma FSH. This procedure utilized an anti-bovine FSH serum furnished by Dr. Cheng. The antigen used to produce this antiserum was a highly purified preparation of bovine FSH with a biological potency of 160 × NIH-FSH-B1 (Cheng, 1976). This same preparation was used for radioiodination (Schanbacher and Ford, 1977) and NIH-FSH-B1 was used as the reference standard. In brief, each tube contained .05 ml of plasma or

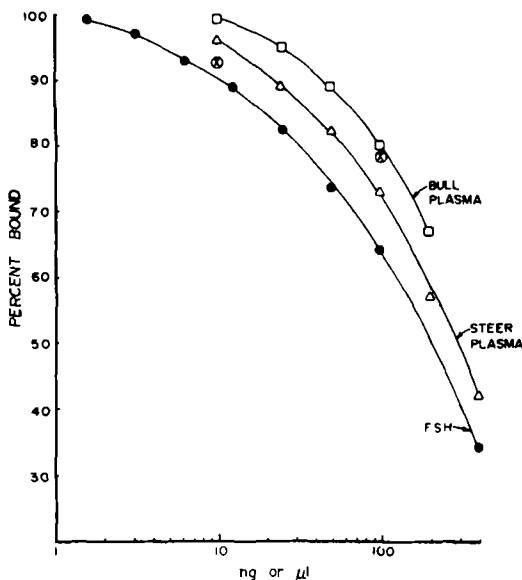


Figure 1. Inhibition curves for bovine FSH (NIH-FSH-B1), steer plasma and bull plasma in the bovine FSH radioimmunoassay. The effects of 10 and 100 ng of bovine LH (NIH-LH-B8), \odot , on the displacement of 125 I-bovine FSH to antibody are shown.

reference standard, .1 ml of antiserum diluted in rabbit gamma globulin (1:60,000) and .05 ml 125 I-labelled FSH tracer. Total volume was brought to .75 ml with .01M phosphate buffer (pH 7.5) containing .14 M NaCl, .001 M EDTA and 1% bovine serum albumin. After 2 days of incubation, .1 ml of diluted sheep anti-rabbit gamma globulin was added to separate free from bound FSH. After an additional 48 hr, 2 ml of cold diluting buffer were added, each tube centrifuged at 600 *g* for 30 min and the precipitate counted in an automatic gamma spectrometer.

Inhibition curves for bull and steer plasma paralleled the reference preparation (NIH-FSH-B1) and further indicated that FSH could be quantitatively determined in 50 μ l of bovine plasma (figure 1). While 10 ng LH resulted in a 7.5% reduction of 125 I-labelled FSH bound to antibody, the maximum concentration of LH in this study (<60 ng/ml or 3 ng/assay tube)

would have no effect on FSH values obtained in this assay. Quantitative recovery ($y = .37 + .90x$; $r = .99$, $P < .001$) was obtained when varying amounts of FSH (0 to 500 ng) were added to 200 μ l of hypophysectomized sheep serum³. This serum contained undetectable amounts of FSH activity (<7.5 ng/ml). All plasma samples were assayed in duplicate within a single assay and the coefficient of variation for all duplicates was less than 14%. Minimum sensitivity was 1.5 ng or 30 ng/ml.

Previously published radioimmunoassay procedures were used to assay in duplicate the jugular and spermatic vein concentrations of testosterone (Schanbacher, 1976) and estrogen (Schanbacher and Ford, 1976). Estrogen concentrations are reported in this study, even though estradiol-17 β was used as the reference standard and the antiserum was of relatively high specificity, because the concentrations of estrogens other than estradiol-17 β in bull plasma are not known. Most important, however was the finding that 250 ng of testosterone did not contribute significantly to the estrogen values obtained in this study. Assays were evaluated and concentration of hormones determined according to the procedures and standards of Rodbard *et al.* (1968). Plasma hormone concentrations were plotted against time relative to saline or GnRH injections and treatment responses were evaluated by regressing the difference between plasma hormone concentrations of GnRH and saline-treated controls on time.

RESULTS

Gonadotropin Releasing Hormone and LH and FSH Release. A rapid increase in plasma concentrations of LH and FSH occurred when mature bulls were administered 500 μ g of GnRH (figure 2). Injection of sterile saline into these same animals was without effect. The mean concentrations of these gonadotropins were increased ($P < .01$) as early as 20 min after the intravenous injection of GnRH and remained increased for approximately 4 hr; maximum concentrations of LH (>30 ng/ml) and FSH (>700 ng/ml) were observed between 40 and 120 min. Contrary to our expectations, episodic release of LH was not observed during the continuous sampling of control bulls. The large increase in plasma concentrations of LH following 500 μ g of GnRH may have masked

³Hypophysectomized sheep serum was kindly furnished by Dr. C. C. Kaltenbach, University of Wyoming, Laramie.

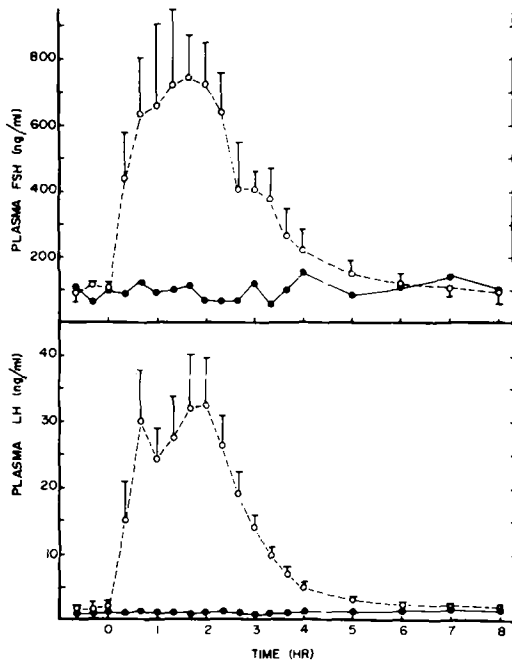


Figure 2. Plasma FSH and LH (Mean \pm SEM) in four Hereford bulls before and after the intravenous injection of saline (●) or gonadotropin releasing hormone (○). Standard errors for the control means are not represented for LH and FSH because all were less than 25% of the mean value.

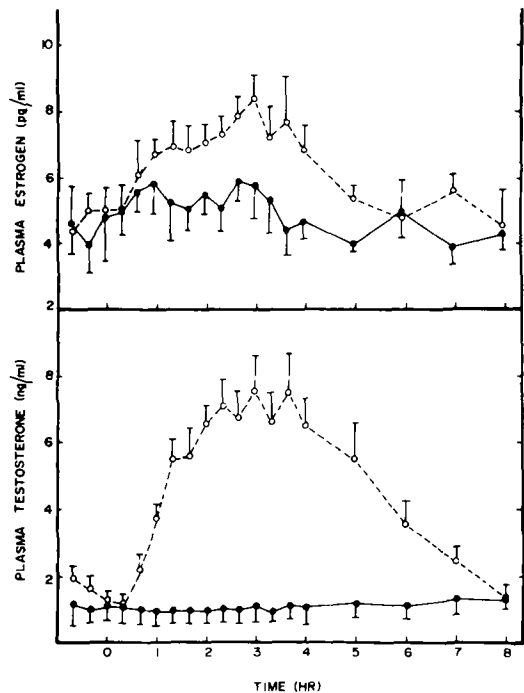


Figure 3. Plasma estrogen and testosterone (Mean \pm SEM) in jugular blood of four Hereford bulls before and after the intravenous injection of saline (●) or gonadotropin releasing hormone (○).

episodic release of LH, however, this was not readily apparent during the 9-hr sampling period. Variation in plasma FSH concentration precluded any interpretation regarding episodic release of this gonadotropin.

Testicular Response to Gonadotropin Releasing Hormone. Systemic plasma concentrations of testosterone and estrogen (figure 3) appear to directly reflect testicular secretion rates because increases in spermatic vein concentrations of these steroids (figure 4) parallel those increases observed in the systemic circulation.

Pretreatment concentrations of testosterone in spermatic vein plasma averaged 30 to 50 ng/ml and increased to greater than 250 ng/ml after the intravenous injection of 500 μ g of GnRH (figure 4). Spermatic vein concentration of testosterone was increased ($P < .05$) within 20 min of treatment and remained above those concentrations found in control animals for 5 hr; testosterone concentrations after this time were not significantly different from pretreatment or control concentrations of testosterone. Testicular secretion of this steroid fluctuated

only slightly in each control animal.

Systemic concentrations of testosterone remained rather constant at approximately 1 ng/ml during the control sampling period for these four animals and increased to 7 ng/ml after the administration of GnRH. This sevenfold increase is comparable with the increase observed in spermatic vein blood. Although an increase in the concentration of testosterone was significant in testicular effluent within 20 min of GnRH exposure, plasma concentrations of this steroid were not significantly increased in the systemic blood until 40 minutes.

Pretreatment concentrations of estrogen in spermatic vein plasma averaged 13 to 16 pg/ml and increased nearly twofold after the injection of releasing hormone (figure 4). Although large between animal variation existed in the four bulls used in this experiment, mean concentrations of estrogen tended to parallel the responses observed for testosterone and regression analysis indicated that the increase in concentrations of estrogen after GnRH was significant. Similar increases ($P < .01$) in systemic concentrations of estrogen were also

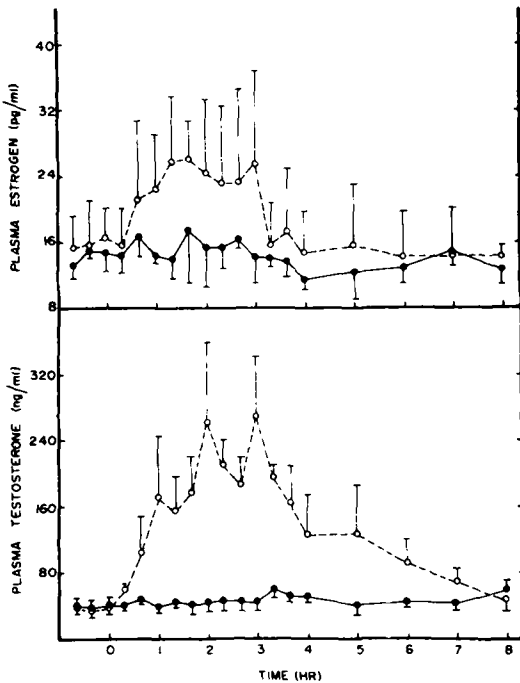


Figure 4. Plasma estrogen and testosterone (Mean \pm SEM) in spermatic vein blood of four Hereford bulls before and after the intravenous injection of saline (●) or gonadotropin releasing hormone (○).

noted after injection of GnRH (figure 3). Concentrations were maximum after 180 minutes. Control samples contained less than 6 pg/ml and averaged only $4.8 \pm .1$ pg/ml during the entire study, considerably less ($P < .01$) than the average concentration ($14.4 \pm .3$ pg/ml) in spermatic vein plasma.

Discussion

Results of this study indicate that intravenous injection of GnRH causes a sudden increase in the circulating concentrations of two gonadotropins, LH and FSH. Although not known for FSH, the height and duration of the LH response depend largely on the dosage of GnRH and route of administration. The 500 μ g of GnRH administered in this study resulted in a large but uniform LH release which conforms to that reported in the literature (Golter *et al.*, 1972; Zolman *et al.*, 1973; Monkonpunya *et al.*, 1974; Mori *et al.*, 1974). Response of FSH to GnRH challenge has been reported to be dose dependent in bulls (Zolman and Convey, 1973); however, considerably more information

regarding release of bovine FSH is available in cows. Gonadotropin releasing hormone (250 μ g) results in a sizeable increase in serum FSH when administered to cows (Akbar *et al.*, 1974) and beef heifers (Kaltenbach *et al.*, 1974). Similarity for the duration of LH and FSH release in cows and heifers was observed in the present study with bulls.

Unlike the findings of some investigators (Katongole *et al.*, 1971), the data presented herein do not support the presence of episodic surges of LH in mature bulls. We have recently confirmed the episodic release pattern of LH which is characteristic of mature rams (Schanbacher and Ford, 1976) and are somewhat surprised by our lack of such a finding in the four mature Hereford bulls used in this study. We cannot determine from these results whether episodic release of LH does not exist in some bulls or whether the frequency or duration of blood sampling in this experiment was inadequate for its detection. It is possible, of course, that the surgical anesthetic interfered with the mechanism responsible for the episodic release of LH. Nonetheless, plasma concentrations of LH and testosterone were within the normal range for mature bulls and GnRH stimulated their secretion. An inability to detect episodic release of FSH in bulls of this study has also been reported by Karg *et al.* (1976).

Increased concentrations of LH in systemic blood of GnRH-treated bulls agree with the findings of Golter *et al.* (1972), Zolman *et al.* (1973), Mongkonpunya *et al.* (1974), Mori *et al.* (1974) and Thibier (1976) and the subsequent increase in plasma testosterone suggests that LH is the major stimulus for the secretion of this steroid. Although FSH may have stimulated testosterone secretion, the specific binding of radioiodinated human chorionic gonadotropin (hCG) to the interstitial cells of the bovine testis (B. D. Schanbacher, unpublished data) and the steroidogenic activity exhibited by these cells (Neaves, 1975) suggest that as in other mammals, GnRH-stimulated testosterone secretion in bulls is mediated by LH.

Additional evidence to support the interaction between gonadotropins and steroidogenesis in bull testes is the sevenfold increase in spermatic vein concentration of testosterone immediately after GnRH-mediated gonadotropin release. The high concentrations of testosterone in testicular tissue (Rawlings *et al.*, 1972) and in spermatic vein (Savard *et al.*,

1961) as compared to the systemic concentration indicate that the primary source of this steroid is some compartment within the bovine testis.

The data presented herein indicate that plasma estrogen concentrations can be regulated in part by the bovine testis. A threefold higher concentration in spermatic vein plasma as compared to systemic plasma indicates that the testis is an important source of estrogen. Amann and Ganjam (1976) have reported comparable data for the bull. These investigators found significantly higher concentrations of estrogen in spermatic venous blood (~ 15 pg/ml) than in systemic blood plasma (~ 11 pg/ml). Furthermore, a large intravenous dosage of hCG (5000 IU) increased substantially the estrogen concentrations in spermatic venous blood (~ 30 pg/ml). Although the increase in plasma testosterone was more substantial, the small but significant increase in plasma estrogen after hCG (Amann and Ganjam, 1976) and GnRH in the present study suggests that estrogen secretion is also regulated by gonadotropins.

The intratesticular site and physiological significance of estrogen biosynthesis and secretion, on the other hand, are not clear. While estrogen may be a product of the interstitial environment (Payne *et al.*, 1976), considerable attention has been given to the Sertoli cell in this respect. Dorrington and Armstrong (1975) have convincing evidence which suggests that rat Sertoli cells can convert testosterone to estradiol and that the synthesis of this steroid is regulated at the level of the aromatizing enzyme system by FSH. Based on this theory, it is quite possible that the increased plasma estrogen in this study could have resulted from an interaction between GnRH-mediated FSH release and increased estradiol synthesis by bovine Sertoli cells. Specific binding of radioiodinated ovine FSH to seminiferous tubular components (B. D. Schanbacher, *unpublished data*) supports this theory; however, additional studies will have to be conducted before any conclusions can be made. Meanwhile, the role of intratesticular estrogen is speculative; perhaps, it is intricately involved with the regulation of spermatogenesis and/or steroidogenesis.

In conclusion, it appears that both testosterone and estrogen are important secretory products of the bovine testis and except for the negative finding with regard to the episodic release of LH and testosterone in intact bulls,

precise coordination between secretions of the hypothalamus, hypophysis and testis appears to follow the same sequential pattern as that determined for males of other species.

LITERATURE CITED

- Akbar, A. M., L. E. Reichert, Jr., T. G. Dunn, C. C. Kaltenbach and G. D. Niswender. 1974. Serum levels of follicle-stimulating hormone during the bovine estrous cycle. *J. Anim. Sci.* 39:360.
- Amann, R. P. and V. K. Ganjam. 1976. Steroid production by the bovine testis and steroid transfer across the pampiniform plexus. *Biol. Reprod.* 15:695.
- Cheng, K. W. 1976. Purification and properties of bovine pituitary follitropin. *Biochem. J.* 159:651.
- Cheng, K. W. 1978. Development and characterization of a homologous radioimmunoassay for bovine follicle-stimulating hormone. *J. Endocrinol.* 77:185.
- deJong, F. H., A. H. Hey and H. J. vanderMolen. 1973. Effect of gonadotropins on the secretion of estradiol-17 β and testosterone by the rat testis. *J. Endocrinol.* 57:277.
- Dorrington, J. H. and D. T. Armstrong. 1975. Follicle-stimulating hormone stimulates estradiol-17 β synthesis in cultured Sertoli cells. *Proc. Natl. Acad. Sci.* 72:2677.
- Echternkamp, S. E., D. J. Bolt and H. W. Hawk. 1976. Ovarian and pituitary hormones in blood of progesterone-treated ewes. *J. Anim. Sci.* 42:893.
- Eiler, H. and C. N. Graves. 1977. Oestrogen content of semen and the effect of exogenous oestradiol-17 β on the oestrogen and androgen concentration in semen and blood plasma of bulls. *J. Reprod. Fertil.* 50:17.
- Ganjam, V. K. and R. P. Amann. 1974. Progesterone and estrogen concentrations in bull reproductive fluids. *Proc. 7th Annu. Meet., Soc. for Study of Reprod.*, Ottawa, p. 169. (Abstr.)
- Golter, T. D., J. J. Reeves, C. C. O'Mary, A. V. Schally and A. Arimura. 1972. Effect of synthetic LH-RH/FSH-RH on serum LH in bulls. *J. Anim. Sci.* 34:914. (Abstr.)
- Haynes, N. B., H. D. Hafs, K. Purvis and J. G. Manns. 1976. Studies on the effect of unilateral and bilateral castration on plasma testosterone and LH levels in the bull. *J. Reprod. Fert.* 46:471.
- Kaltenbach, C. C., T. G. Dunn, T. E. Kiser, L. R. Corah, A. M. Akbar and G. D. Niswender. 1974. Release of FSH and LH in beef heifers by synthetic gonadotropin releasing hormone. *J. Anim. Sci.* 38:357.
- Karg, H., T. Gimenez, M. Hartl, B. Hoffmann, E. Schallenberger and D. Schams. 1976. Testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in peripheral plasma of bulls: Levels from birth through puberty and short term variations. *Zbl. Vet. Med.* 23:793.
- Katongole, C. B., F. Naftolin and R. V. Short. 1971. Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. *J. Endocrinol.* 50:457.
- Kley, H. K., E. Nieschlag, W. Wiegmann and H. L. Kruskemper. 1976. Oestrone, oestradiol and

- testosterone in normal and hypogonadal men following LH-RH or HCG stimulation. *Acta Endocrinol.* 81:616.
- Mahoudeau, J. A., J. C. Valcke and H. Bricaire. 1975. Dissociated responses of plasma testosterone and estradiol to human chorionic gonadotropin in adult men. *J. Clin. Endocrinol. Metab.* 41:13.
- Mongkonpunya, K., H. D. Hafs, E. M. Convey, W. D. Oxender and T. M. Louis. 1974. Luteinizing hormone release by gonadotropin releasing hormone before and after castration in bulls. *Proc. Soc. Exp. Biol. Med.* 147:873.
- Mori, J., T. Endo, T. Takahashi and J. Masaki. 1974. Release of LH after administration of an analog of synthetic LH-RH in cattle. *Theriogenology* 1:177.
- Neaves, W. B. 1975. Leydig cells. *Contraception* 11:571.
- Niswender, G. D., L. E. Reichert, Jr., A. R. Midgley, Jr. and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* 84:1166.
- Payne, A. H., R. P. Kelch, S. S. Musich and M. E. Halpern. 1976. Intratesticular site of aromatization in the human. *J. Clin. Endocrinol. Metab.* 42:1081.
- Rawlings, N. C., H. D. Hafs and L. V. Swanson. 1972. Testicular and blood plasma androgens in Holstein bulls from birth through puberty. *J. Anim. Sci.* 34:435.
- Rodbard, D., P. L. Rayford, J. A. Cooper and G. T. Ross. 1968. Statistical quality control of radioimmunoassays. *J. Clin. Endocrinol. Metab.* 28:1412.
- Savard, K., N. R. Mason, J. T. Ingram and F. X. Gassner. 1961. The androgens of bovine spermatic venous blood. *Endocrinology* 69:324.
- Schanbacher, B. D. 1976. Rapid chromatography for quantitation of radioimmunoassayable 5 α -17 β -ol-3-one and testosterone in ram, bull and boar serum. *Endocrine Res. Comm.* 3:71.
- Schanbacher, B. D. and J. J. Ford. 1976. Seasonal profiles of plasma, LH, testosterone and estradiol in the ram. *Endocrinology* 99:752.
- Schanbacher, B. D. and J. J. Ford. 1977. Gonadotropin secretion in cryptorchid and castrate rams and the acute effects of exogenous steroid treatment. *Endocrinology* 100:387.
- Smith, O. W., K. Mongkonpunya, H. D. Hafs, E. M. Convey and W. D. Oxender. 1973. Blood serum testosterone after sexual preparation or ejaculation, or after injections of LH or prolactin in bulls. *J. Anim. Sci.* 37:979.
- Thibier, M. 1975. Variations circadiennes de la LH plasmatique chez le jeune taurillon. *Annales d'Endocrinologie* 36:205.
- Thibier, M. 1976. Effect of synthetic gonadotropin-releasing hormone (Gn-RH) on circulating luteinizing hormone (LH) and testosterone in young post-pubertal bulls. *Acta Endocrinol.* 81:635.
- Weathersbee, P. S. and J. R. Lodge. 1976. Serum testosterone and estrogen concentrations in the Holstein-Friesian bull after successive ejaculations. *Amer. J. Vet. Res.* 37:465.
- Zolman, J. and E. M. Convey. 1973. GnRH: Effect on serum FSH and androgens in bulls. *J. Anim. Sci.* 37:334. (Abstr.)
- Zolman, J., E. M. Convey, J. H. Britt and H. D. Hafs. 1973. Release of bovine luteinizing hormone by purified porcine and synthetic gonadotropin releasing hormone. *Proc. Soc. Exp. Biol. Med.* 142:189.